REMARKS

This amendment is in response to the Non-Final Office Action mailed on February 18, 2004. Claims 1-46, 48-57, 62-65, and 74-112 have been cancelled without prejudice. Claims 47, 58, 60, 61, 66, 68 and 70 are currently amended. New claims 113-119 have been added. Claims 47, 58-61 and 66-73 and 113-119 are now pending. Reconsideration is respectfully requested in view of the above amends and the following remarks.

I. Rejection Under 35 U.S.C. 112, Second Paragraph

The Examiner has rejected claims 47, and 58-73 under 35 U.S.C. §112, second paragraph, as being indefinite.

Applicants amend independent claim 1 to specify a method of inducing death in cancer cells that express an apoptosis-mediating receptor. By introducing an adenoviral vector encoding an apoptosis signaling ligand into cancer cells that express an apoptosis mediating receptor, the cancer cells are induced to die through the specific binding interaction between the apoptosis-signaling ligand and the apoptosis-mediating receptor. As suggested by the Examiner, Applicants amend claim 1 to specify the cell death is induced by specific binding of the apoptosis-signaling ligand to the apoptosis-mediating receptor. Withdrawal of the rejection under 35 U.S.C. §112, second paragraph is therefore respectfully requested.

II. Rejection Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 47, and 58-73 under 35 U.S.C. 112, first paragraph. Specifically, the Examiner states that the specification, while being enabling for *in vitro* use, FasL-Fas mediated cell death and the vector rAD/FasL-GFP$_{TETd}$, does not reasonably provide enablement for *in vivo* use, cell death via expressing any ligand in any ligand-receptor-mediated apoptosis and expression of FasL using any expression vectors.

Independent claim 47 as amended specifies a method for inducing death of cancer cells by introducing to the cancer cells an adenoviral vector encoding an apoptosis-signaling ligand. The

Examiner's rejection of the claims for lack of enablement is based on the Examiner interpreting the claims as covering a method of curing cancer by gene therapy. While curing cancer by gene therapy is a potential utility of the claimed invention, this particular utility is not being claimed. Rather, the utility claimed is inducing cell death in cancer cells through specific binding interactions between the apoptosis-signaling ligand to the apoptosis-mediating receptor. Since enablement of a claim under 35 USC §112, First Paragraph must be measured against the utility that is being claimed and not all potential but unclaimed utilities, the Examiner's rejection for lack of enablement is improper, unsupported, and should be withdrawn.

The Examiner also requires Applicants to show a use of the claimed method *in vivo* in order to satisfy the enablement requirements. However, pursuant to MPEP 2164.02 compliance with the enablement requirement of first paragraph, does not turn on whether an example is disclosed. An example may be "working" or "prophetic." An applicant need not have actually reduced the invention to practice prior to filing. In *Gould v. Quigg*, 822 F.2d 1074, 1078, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987). In the specification Applicants describe how to construct the expression vector encoding an apoptosis-signaling ligand (e.g., Fas Ligand, Bax, Bad, Bak, and Bik, page 9, lines 4-11) and how to introduce the expression vector into cancer cells to induce cell death, *inter alia*, starting at page 17, line 26 throughout pages 18-21, and in Example 3 at page 34. In view of the ample description in the specification, Applicants submit that the disclosure of the application is sufficiently enabling to one of ordinary skill in the art under 35 USC §112, First Paragraph.

Nonetheless, Applicants submit herewith an excerpt (Exhibit A) from U.S. Patent Application Serial No. 09/656,779 (a continuation-in- part of this application) that shows the effectiveness of an adenoviral vector encoding Fas ligand in induction of cancer cell death and tumor regression in breast cancer xenografts *in vivo*. These experimental results further demonstrate that by using the method described in the instant application that one could induce death of cancer cells in vitro and in vivo and thus remove any basis the Examiner might have to doubt enablement.

For the above reasons provided, Applicants respectfully request that the Examiner's rejection under 35 USC 112, First Paragraph be withdrawn.

III. Rejection Under 35 U.S.C. 103(a)

The Examiner has rejected claims 47 and 58-73 under 35 U.S.C. 103(a) as being unpatentable over Larregina et al. (Gene Therapy, Jan. 1998, 5:563:568) in view of Arai et al. (Proc. Natl. Acad. Sci. Dec. 1997, 94:13862-7).

Independent claim 47 as amended specifies a method for inducing death of **cancer cells** by introducing to the cancer cells an adenoviral vector encoding an apoptosis-signaling ligand. As described above in response to the rejection under 35 USC 112, First Paragraph, Applicants have demonstrated that by using the method described in the instant application that one could induce death of cancer cells in vitro and in vivo.

In contrast, Larregina et al. discloses a method of generating adenoviral vectors expressing FasL in human embryonic kidney 293 cells which are not cancer cells. Larregina et al. tried to solve the problem of massive cell death of 293 cells after cotransfection with a shuttle plasmid encoding the mouse FasL and a plasmid containing the genome of adenovirus type 5 with deletion in the E1-E3 regions. *See* Abstract. It is well known in the art that 293 cells are a immortalized human cell line which is routinely used for producing higher titers of recombinant adenovirus for various applications. Page 566, column 2, 3rd paragraph, lines 1-4. Larregina et al. teaches that in order to increase the titers of the adenovirus expressing FasL, different approaches could be used to **circumvent FasL-induced apoptosis of 293 cells**, including 1) silencing the expression of FasL during the development of the viral vectors in 293 cells; and 2) incubating 293 cells either with inhibitors of FasL-induced apoptosis, etc. Page 566, column 2, 3rd paragraph, lines 11-15. Larregina et al. teaches a method of producing recombinant adenovirus expressing FasL by **inhibiting apoptosis induced by FasL in 293 cells**. Therefore, this reference not only fails to teach using FasL to induce cell death but also teaches away from the claimed method of using FasL expressed by an adenoviral vector to **promote cancer cell death** through interaction of Fas and FasL.

The secondary reference cited, Arai et al., fails to supply the claim element missing in the primary reference, Larregina et al. Arai et al. teaches transferring FasL into CT26 colon cancer cells that do not express Fas. *See* Abstract. Further this reference teaches transferring FasL via a

plasmid that uses the CMV promoter and stably transduce CT26 cell lines by electroporation. Page 13862, column 2, 3rd paragraph, under "Generation of Stably Transduced FasL-Expressing Cell Line". Thus Arai et al. also fails to teach the claimed method of inducing death of cancer cells via specific interaction of an apoptosis mediating-receptor (e.g., Fas) and an apoptosis-signaling ligand (e.g., FasL) that is expressed by an adenoviral vector.

To establish a prima facie case of obviousness, the Examiner bears the burden of proving 1) the prior art reference (or references when combined) must teach or suggest all the claim limitations; 2) the prior art contains a suggestion or motivation to combine the prior art references in such a way as to achieve the claimed invention; and 3) one of ordinary skill in the art at the time the invention was made would have reasonable expectation of success of the claimed invention. *In re Vaeck*, 947 F. 2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991); *In re O'Farrell*, 853 F. 2d 894, 903-904, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988); and *In re Dow Chem.*, 837 F. 2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

As discussed above, none of the cited references teaches or suggests the claimed method of inducing death of cancer cells via specific interaction of an apoptosis mediating-receptor (e.g., Fas) and an apoptosis-signaling ligand (e.g., FasL) that is expressed by an adenoviral vector. Absent some suggestion of modifying Larregina et al. to arrive at the claimed method in the cited references, a prima facie case of obviousness has not been established. Withdrawal of the rejection under 35 U.S.C. §103(a) is therefore respectfully requested.

CONCLUSION

Applicants earnestly believe that the application is in condition for allowance and respectfully solicit the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to Deposit Account No. 23-2415 (Attorney Docket No. 22488-710).

Respectfully submitted,

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EXHIBIT A

METHOD COMPOSITION FOR TREATING TUMORS BY SELECTIVE INDUCTION OF APOPTOSIS

Inventors: Jian-yun Dong and James S. Norris

BACKGROUND OF THE INVENTION

REFERENCES TO PARENT AND CO-PENDING APPLICATIONS

This application claims the priority of U.S. Provisional Application No. 60/107,363 entitled "Method for Treating Tumors Using Fas-Induced Apoptosis", filed on November 6, 1998; PCT Application No. PCT/US99/26221 entitled "A Method of Treating Tumors Using Fas-Induced Apoptosis", filed on November 5, 1999 and published on May 18, 2000, International Publication No. WO 00/27883; and U.S. Patent Application Serial No. 09/600,521 entitled "Method and composition for Treating Tumors Through Fas Ligand-Induced Apoptosis", filed on July, 14, 2000. The above applications are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to compositions and methods for inducing programmed cell death (apoptosis) in cancer cells, and more particularly, relates to compositions and methods for treating tumors by using expression vectors that expresses an apoptosis-signaling ligand such as Fas ligand (Apo-1 ligand) and TRAIL (Apo-2 ligand). Expression of the apoptosis-signaling ligand induces apoptosis in cells expressing an apoptosis-mediating receptor such as Fas (receptor for Fas ligand), and DR4 or DR5 (receptor for TRAIL).

Virus dosages of $5x10^9$, $1x10^{10}$, and $5x10^{10}$ in a constant 400ul volume are used: one set of 2 dogs receives Ad/CMV-LacZ at $5x10^{10}$ pfu to allow histochemical monitoring of viral spread. Dogs are monitored closely the first 72 hours for any signs of distress. Feces is collected and analyzed for viral shedding by PCR. Urine is also collected by foley catheter and assayed on 293 cells for shed virus and by PCR. At day 7 (2 dogs per viral dose) are euthanized with sodium pentobarbital and processed as described. (Andrawiss et al. Prostatic Can. Prostatic Dis. 2:25-35, 1999). Samples of all tissues are frozen in OCT while the remainder are either fixed and processed for histology (tunel, immunohistochemistry), or stored frozen at -80°C for DNA extraction and PCR using viral-specific primers. Expression of LacZ is examined in the Ad/CMV-LacZ group to monitor systemic viral spread.

Example 4: Intratumoral Introduction of Ad/GFP-FasL^{TET} Suppresses Breast Tumor and Brain Tumor Growth in Mice

In this experiment, we implanted 10^6 MCF-7 cells bilaterally into Balbc nu/nu mice (Figure 6). When tumor sizes reached 5 mm in diameter, we infused at 15 μl per minute, 2 X 10^9 pfu Ad/GFP-FasL^{TET} into the tumors on the right side of the mouse or 2 X 10^9 pfu Ad/LacZ into the left side, over a period of 10 minutes using a Harvard infusion pump. At three weeks post-injection, all tumors injected with Ad/FasL-GFP^{TET} exhibited about 80-100% regression of the tumor in comparison with the control-treated tumor. In particular, in four of the six mice treated, most of the tumor masses disappeared after one injection (indicated by yellow arrows). In the other two of the six mice, suppression of tumor growth wad greater than 80% (indicated by black arrows) in comparison to tumors on the control side of the same mice. In contrast, all tumors injected with Ad/LacZ grew to about 2 cm in diameter at three weeks after implantation. Histological analysis of the residual tumors in some of the mice showed only infiltrating immune cells and fibroblasts with no apparent cancer cells remaining. This demonstrates that FasL-induced apoptosis

may be used as a novel treatment for breast cancer.

Similarly, we implanted 10^6 SF767 cells bilaterally into Balbc nu/nu mice. When tumor sizes reached 5 mm in diameter, we infused at 15 μl per minute, 2 X 10^9 pfu Ad/GFP-FasL^{TET} into the tumors on the right side of the mouse or 2 X 10^9 pfu Ad/LacZ into the left side, over a period of 10 minutes using a Harvard infusion pump. Tumor suppression was about 80-100% in treated tumors as compared to untreated tumors. In contrast, all tumors injected with Ad/LacZ grew to about 2 cm in diameter at three weeks after implantation. This demonstrates that FasL-induced apoptosis may be used as a novel treatment for brain cancer.

Example 5: Comparison of sensitivities of cancer cells to FasL- and TRAIL-induced apoptosis in vitro

In this example, sensitivities of different cancer cell lines (derived from prostate, cervical and liver cancers) to FasL- and TRAIL-mediated apoptosis were compared in vitro.

An expression vector for TRAIL, Ad.TRAIL/GFP^{TET}, was used to express TRAIL in these cancer cells. The construction of Ad.TRAIL/GFP^{TET} is illustrated in Figure 3. Similar to Ad/FasL-GFP^{TET}, this vector contains a transactivator driven by the CMV promoter in the E1 region, and the TRAIL-IRES-GFP expression cassette under the control of the TRE promoter in the E4 region, so that the expression of both TRAIL and GFP can be regulated by the addition of doxicycline to the culture media. The internal ribosome entry site (IRES) of the encephalomyocarditis virus allows expression of two genes from the same mRNA transcript. Although the GFP is not fused to the apoptotic protein TRAIL, its expression is correlated with that of TRAIL. Since TRAIL is in front of the GFP and the IRES sequence, the level of its expression should be several folds higher than GFP. Liu et al. (2000) "Generation of mammalian cells stably expressing multiple genes at predetermined levels" Anal. Biochem. 280:20-28. This will assure high levels of TRAIL expression in cells that

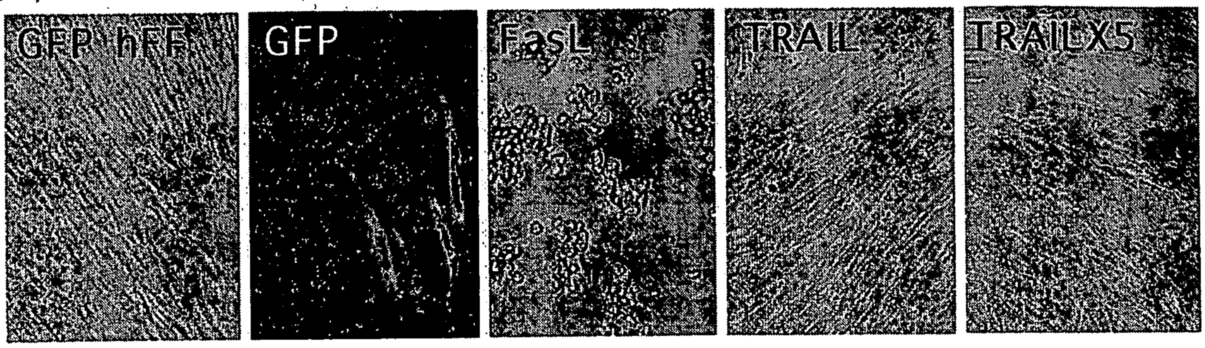

Fig. 5. ***TRAIL expression do not induced apoptosis in untransformed fibroblasts.*** *To determine that if TRIAL expression will induce apoptosis in normal cells, low-passage human foreskin fibroblasts were infected with AdGFP, AdFasL-GFP^{TET}, and AdTRAIL/GFP^{TET} at MOI about 10. The bright-field veiw shows the normal morphology of fibroblasts transduced with AdGFP (panel GFP hFF). Fibroblasts demonstrated poor infectability by adenovirus as shown by the low number of GFP expression cells (GFP). However, these cells are highly sensitive to FasL induced apoptosis (FasL). In contrast, no apparaent apoptosis can be observed in TRAIL transduced cells (TRAIL), even at five fold of the MOI (MOIX5).*

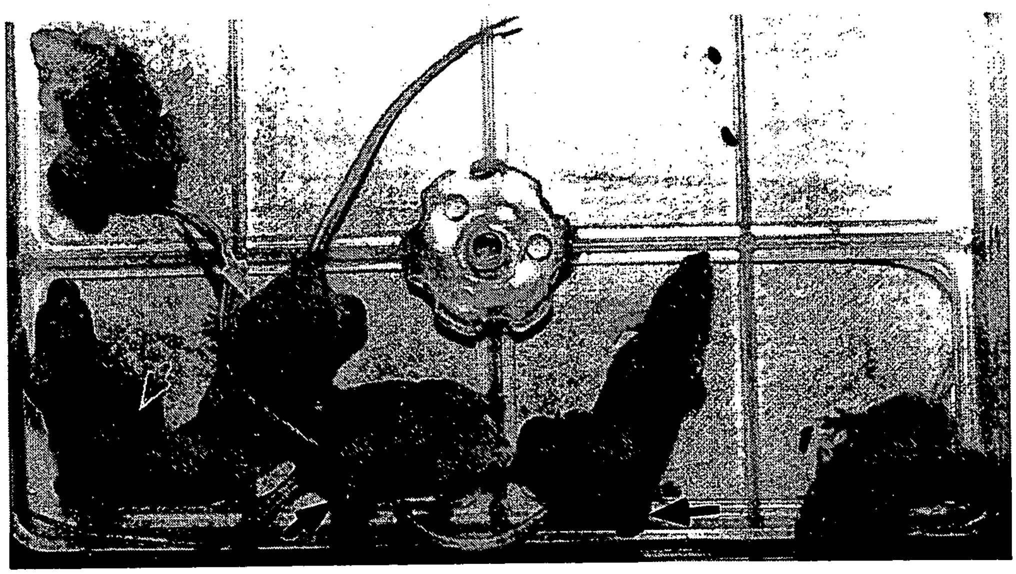

Figure 6. Inducing Apoptosis of Implanted Breast Tumors in Nude Mice. *Equal numbers of breast cancer cells were implanted in each side of six mice. Tumors on the right side of the mice were injected with rAd/FasL-GFP^{TET} vector, and on the left, injected with a control vector rAd/LacZ. In four of the six mice, most of the tumor masses disappeared after one injection (indicated by yellow arrows). In two of the mice, suppression of tumor growth was greater than 80% (black arrows) in comparison to tumors on the control side.*